6th Annual International IEEE EMBS Conference on Neural Engineering
San Diego, California, 6 - 8 November, 2013

Drug Eluting Coating for 3D Parylene Sheath Electrode*

Curtis Lee, *Student Member, IEEE,* Lawrence Yu, Jonathan T. W. Kuo, Brian Kim, Tuan Hoang, and Ellis Meng, *Senior Member, IEEE*

***Abstract*— Two methods for incorporating drug eluting coatings consisting of Matrigel (MG) loaded with dexamethasone (DEX) onto the Parylene sheath electrode (PSE) were developed and compared. The purpose of the coatings is to reduce the immune response evoked by tissue damage during electrode insertion into the cortex and subsequent sustained aggravation of tissues by the implant. Parylene surfaces are hydrophobic and repel MG, therefore, both physical and chemical methods were investigated to disrupt surface tension and increase surface energy to facilitate even coating onto the PSE. A gelling step was also investigated to improve loading of coating onto PSE. Spectrophotometry was used to measure the amount of DEX loaded onto the PSE. Loading of up to 563 ng of DEX was achieved by using a combination of surface energy modification and coating gelling, whereas sonication assisted coating methods loaded 205 ng.**

I. Introduction

Intracortical electrodes serve an important role in the acquisition of extracellular electrical activity in order to better understand the function of the brain and drive prosthetic devices such as robotic limbs. A common approach is to fashion intracortical electrodes from rigid tines or planar shafts made from either metal, such as stainless steel or tungsten, or silicon, respectively [1]. Implantation of these electrodes induces an immediate immune response to the stab wound injury and a sustained response associated with continued aggravation by micromotion combined with mechanical mismatch of the electrodes with tissue. The immune response results in a glial scar around the electrode and neuronal retraction, which increases the distance between the neuron and recording site, raises the effective electrode impedance, and has been correlated to signal attenuation [2]. One strategy used to reduce inflammation around the electrode site and increase the signal to noise ratio of recordings is to coat neural electrodes in either adhesive molecules to stimulate attachment of cells to the surface of the array [3], immunosuppressants to reduce the immune response [4], or neurotrophic factors that support cell viability and encourage growth and differentiation of neurons towards the electrode [5].

* This work was sponsored by the Defense Advanced Research Projects Agency (DARPA) MTO under the auspices of Dr. Jack Judy through the Space and Naval Warfare Systems Center, Pacific Grant/Contract No. N66001-11-1-4207.

C. Lee, L.Yu, J. T. W. Kuo, B. Kim, T. Hoang and E. Meng are with the Department of Biomedical Engineering at the University of Southern California, Los Angeles, CA 90089 USA (corresponding author: 213-740-6952; e-mail: ellis.meng@usc.edu).

Another strategy is to change the physical design of the neural electrode such as Kennedy's neurotrophic electrode (NE) and the Parylene Sheath Electrode (PSE) developed at the Biomedical Microsystems Lab. Kennedy's NE consists of a glass cone made from the tip of a patch clamping pipette with microwire electrodes—deinsulated at the tip—manually affixed to the inside of the cone [6]. Kennedy's NE has been used to collect signals in human subjects for 5 years [7], but because the cone is assembled by hand out of glass, the flexibility of its design and scale of its production is limited. Initially, Kennedy threaded an autologous section of sciatic nerve through the cone to encourage neurons to grow into the cones, next to the electrodes [6], but later substituted the nerve with either Matrigel (MG), nerve growth factor, or a combination of the two [8].

MG is an extract from the Engelbreth-Holm-Swarm mouse sarcoma that is rich in extracellular matrix protein (adhesion molecules) such as laminin, collagen, and entactin, and contains growth factors such as epidermal, nerve, and fibroblast growth factors [9]. MG is routinely used in cell culturing to induce differentiation and produce realistic morphologies of cell structures *in vitro* and *in vivo* to deliver and support stem cells [10], and assay anti-angiogenesis drugs [11].

The PSE is similar in principle to Kennedy's NE, consisting of a cone shaped sheath lined with electrodes on the inside and outside [12] (Fig. 1). However, the PSE is micromachined rather than handmade, which allows greater flexibility of design, including the shape of the sheath and number of electrodes, and is amenable to mass production. The PSE is manufactured from the biocompatible (USP Class VI) polymer Parylene (which is also used to coat other FDA approved implantable devices such as pacemakers, cochlear implants, and controllers for deep brain stimulation). The lower modulus of Parylene compared to metals, silicon, glass, and other polymers may reduce damage associated with micromotion, while the sheath structure accommodates neural tissue ingrowth towards electrode recording sites for improved chronic acquisition of neuronal signals. The thin film Pt electrodes are deposited on the Parylene surface; platinum is an inert metal commonly used as an electrode in neural interfaces and is biocompatible.

This study presents and compares two methods used to coat the PSE with MG loaded with dexamethasone (DEX), a powerful immunosuppressant shown to reduce the immune response when administered intracranially [13]. MG was selected because of its success in Kennedy's NE and its ability to cause neurons to differentiate *in vitro* and to

support developing neurons *in vivo* [10, 14]. Scanning electron microscopy (SEM) was used to determine distribution of MG on the PSE and confirm uniformity of the coating. The uniformity ensures that MG is presented to adjacent tissue and can serve an adhesion molecule to promote neuronal attachment to the PSE. Spectrophotometry was used to measure DEX loading onto the PSE in order to ascertain MG's effectiveness as a drug eluting matrix.

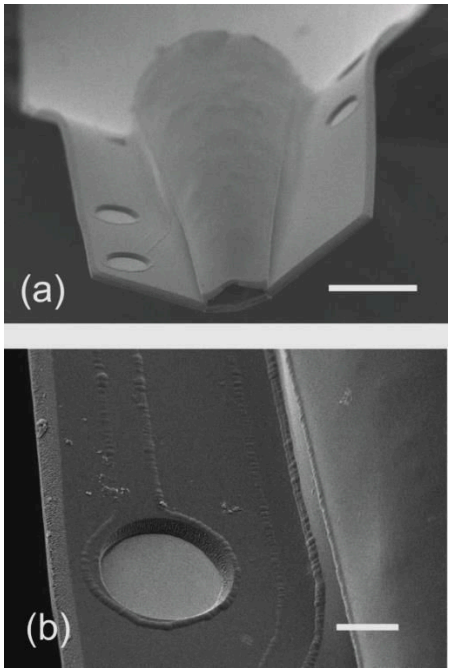

Figure 1. (a) SEM image of uncoated PSE viewed from sheath tip (scalebar = 100 µm). Electrodes flanking the central sheath are visible. (b) Zoomed in view of a single uncoated electrode site (scalebar = 25 µm).

II. Materials and Methods

Coatings were based on MG (BD Biosciences, San Jose, CA) diluted with 1× phosphate buffered saline (PBS) at a ratio of 790µL:210µL. This matrix was used as a drug-eluting coating. Specifically, water soluble DEX (D2915 Sigma Aldrich, St. Louis, MO) was added to the MG:PBS solution, resulting in a final concentration of 2 mg/mL.

A. Coating Methods

At 4 °C MG is a liquid which behaves similar to water, but which gels quickly when brought to room temperature. Being 90% water, MG will dehydrate if left uncovered. These properties make coating MG onto surfaces challenging. When the PSE was submersed into liquid MG, trapped gasses formed bubbles on both openings of the sheath upon submersion into cold MG solution, preventing MG from entering the sheath interior.

Several methods were used to eliminate these bubbles, such as pulling a micro-filament through the sheath after dropping liquid MG onto the PSE and using O_2 plasma to increase the surface energy of the Parylene sheath. These methods were abandoned because of the non-uniform penetration of MG when using the filament and its technical complexity. In the case of O_2 plasma, coating would wick up the PSE cable resulting in uneven coating. Two methods, sonication and surface modification using the positively charged molecule poly-D-lysine (described in more detail below), were further investigated after preliminary experiments showed these methods successfully pulled MG into the sheath and resulted in more uniform and controlled coatings.

1) Sonication: Ultrasonic vibrations in liquids create cavitation bubbles that agitate liquids and break surface tension. To apply ultrasonic vibrations to the sheath, the sheath was submersed into a vial containing the coating solution and then the vial with the PSE was placed into an ultrasonic bath (Branson Ultrasonics, Danbury, CT) at 4 °C for 5 min. The PSE was then removed from the coating solution and allowed to gel and dehydrate at room temperature for at least 5 min.

2) *Surface modification:* The positively charged poly-D-lysine (PDL) molecule was used to form a hydrophilic monolayer on the Parylene surface by first dipping the sheath into 70% ethanol solution for 10 s, and then immediately transferring the ethanol filled sheath to a 100 µL/mL solution of PDL (P6407, Sigma Aldrich, St. Louis, MO) and soaking for 1 hour. The PSE was then removed from the PDL solution and rinsed by soaking in triple distilled water (EMD Millipore, Billerica, Massachusetts) for 45 s and then allowed to dry at room temperature. The PSE was then placed on a polystyrene surface, and a micropipette was used to apply a 10 µL droplet of the coating solution to the PSE. The PSE was then covered with a lid (to prevent evaporation) and placed into an oven at 55 °C for 5 min to cause the MG to gel. The coated PSE was then removed from the droplet and placed (uncovered) back into the oven at 55 °C to remove liquid from the coating to facilitate handling and decrease the cross section of the coating.

B. SEM

Scanning electron microscope (SEM) images (7001, JEOL, Peabody, MA) were used to view the morphology of the coating. PSEs were sputtered with Au prior to imaging. Images were taken using a 5 kV beam to minimize charging effects of the polymer during viewing.

C. Drug Loading Measurement

To measure drug loading, the coated PSEs (6 PSEs that had been sonicated and 4 PSEs coated using surface modification) were soaked in 100 µL of PBS for 2 h. The eluent was then scanned using a microplate reader (Epoch, Biotek, Winooski, VT). The absorbance of the eluent was compared to reference solutions of known concentrations of DEX to determine the volume of DEX. Reference solutions were made by diluting DEX in PBS (with and without MG) to a given concentration (e.g. 200 µg) and then serially diluting this solution until the absorbance was similar to 1× PBS.

An additional soak test was run with four PSEs coated using the surface modification method where the PSEs were removed at specific intervals (15 min, 30 min, 1 h, 2 h). Between intervals, samples soaking in microwells were covered in plastic to prevent evaporation.

III. Results and Discussion

A. SEM evaluation of coating methods

Both Parylene and MG are essentially transparent and therefore, SEM imaging was used to visualize the coating on the PSE. SEM images of the sonicated PSE reveal MG coating that is thickest on either side of the sheath, but thinner (or not present) towards the outer edges of the polymer flaps flanking the sheath (Fig. 2a). The PSE coated using the surface modification approach is largely conformal; there are no distinguishable thick and thin regions, except for the area directly above the large end of the sheath (Fig. 2b). The edges of electrodes on the PSE coated using the sonication method (Fig. 2c) are less distinct and suggest that a thicker coating over electrodes compared to the surface modification approach (Fig. 2d).

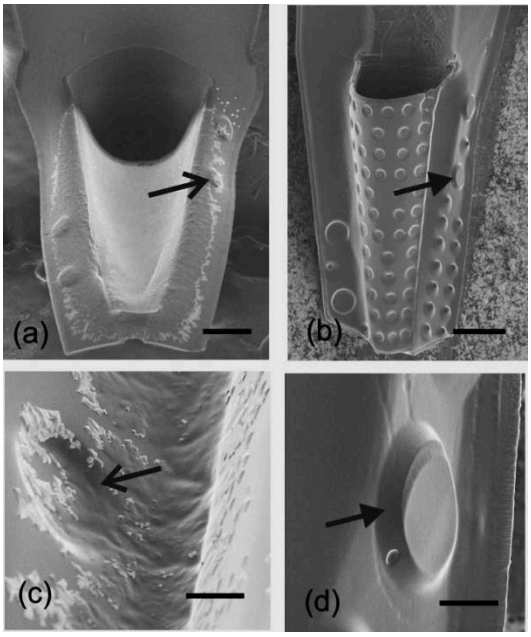

Figure 2. SEM images of coated PSEs using (a & c) sonication and (b & d) surface modification approaches (scalebar on a & b = 100 µm; scalebar on c & d = 20 µm). (c & d) Enlarged images of specific electrodes indicated by arrows in a & b. Note that two distinct PSE types are shown. In b & d, a newer PSE with perforations (smaller diameter than electrodes) through the substrate and sheath is shown. The perforations are completely sealed by the MG-based coating.

B. Drug Loading Measurement

To determine the amount of drug that could be loaded onto a PSE, a calibration curve was made with DEX dissolved into PBS (Fig. 3). This curve was compared to dilutions of DEX loaded MG coating diluted into PBS to confirm that the proteins in the MG did not affect the absorbance of the solution at 242 nm.

The absorbance of the calibration solutions with MG was higher than the absorbance of the PBS/DEX solutions at wavelengths lower than 242 nm. However, at 242 nm the absorbance of the two solutions were very similar, with the exception that at 200 µg/mL, where the absorbance of the MG solution was slightly higher than the solution without MG. These data indicate that although absorbance measurements of solutions containing DEX at 242 nm are typically selective [15], high concentrations of proteins may result in an increase in absorbance at 242 due to a broadband increase of absorbance.

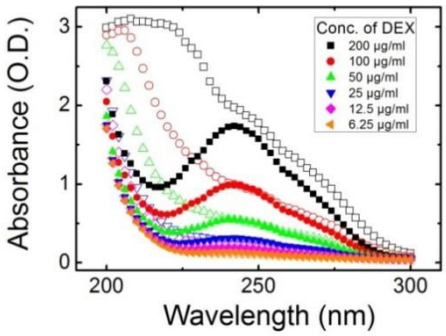

Figure 3. Spectrophotometric scans of DEX (filled symbols) and DEX mixed with MG (outlined symbols) from 200-300 nm showing that proteins from MG do not shift absorbance at 242 nm.

The absorbance of the eluent from PSEs coated with sonication was less than the absorbance measured from PSEs coated using the surface modification method even though there were more PSEs coated with sonication than with surface modification (Fig. 4). The absorbance of the sonicated PSEs corresponds with 205 ng of DEX being contained on each PSE, whereas the absorbance of the eluent from surface modified PSEs corresponds to 563 ng of DEX per PSE. The surface modification method is able to hold more coating because there is a larger surface area available to be coated (when PSE is sonicated, the coating is thicker at the junction of the sheath and the substrate and is thinner approaching the edges (Fig. 2a)) and the additional gelling step which increases the viscosity of the MG and allows a larger amount of coating to adhere to the PSE during the dehydration step.

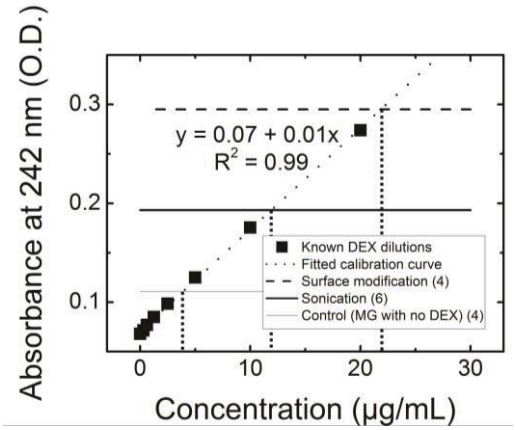

Figure 4. Comparison absorbance of eluent from 6 PSEs coated using sonication (solid horizontal line) compared with 4 PSEs coated using the surface modification method (long dashed horizontal line). Dotted diagonal line is a fit to known concentrations of DEX. *X*-axis intercept between fitted line and absorbance of eluent is concentration, which is directly proportional to mass of DEX loaded onto PSE. Numbers in parentheses denote numbers of PSEs in each study.

When the absorbance was measured at specific time intervals, the highest concentration of DEX was observed after the first time period, and subsequently gradually

decreased over time (Fig. 5), suggesting that nearly all the DEX was eluted during the first 15 min. The gradual decline in absorbance over time may be explained by some evaporation or liquid lost as the PSEs were removed from the wells during scans and imperfect sealing of the microwell during the two hour duration of the experiment. Different measurement techniques will have to be used to improve the time resolution of drug release kinetics and eliminate evaporation of solution from the test wells.

Previous studies have shown both acute (< 2 weeks) and chronic (> 2 weeks) reponses to inserted neural electrodes [16]. The coatings presented here will deliver DEX to the implantation site and improve the acute response, but slower release coatings must be developed to modulate the chronic response because the halflife of DEX in the brain has been determined to be 16 h [17]. However, effects of MG may persist as it has been shown to endure in the brain over the course of several weeks [18].

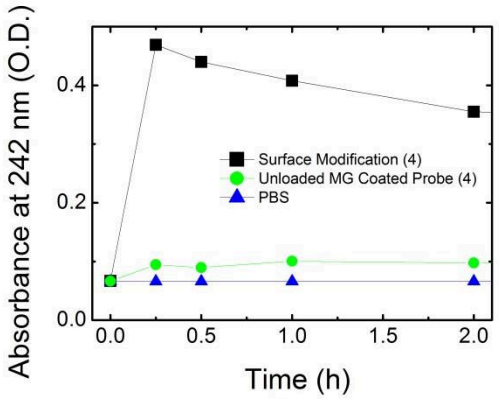

Figure 5. Absorbance at 242 nm of eluent from 4 PSEs coated with DEX loaded MG using the surface modification method measured at various time points over 2 hours. Results are compared to eluent of 4 PSEs coated in MG not loaded with DEX and a microwell containing only PBS.

IV. Conclusion

This paper presents several strategies used to coat the three dimensional PSE with a drug eluting coating based on MG. Using sonication to break the surface tension across the top of the sheath helped the coating to enter the sheath in order to coat the inner electrodes as well as the outer electrodes. However, coatings achieved were not uniform across the PSE surface. In a second method, PDL was first absorbed onto the surface of the Parylene to render the surface hydrophilic and facilitate transport of coating to the sheath interior. SEM images show a more uniform coating on the surface treated PSE than on the sonicated PSE.

Spectrophotometry measurements demonstrated that eluent from PSEs that were surface treated contained more DEX than PSEs that were sonicated. Measuring the absorbance of the eluent of the PSE after specific time periods showed a release of the DEX within 15 min of soaking suggesting that this coating is best suited for managing inflammation in acute studies.

Acknowledgment

This work was funded in part by the University of Southern California Viterbi Fellowship (CL). The authors would like to thank the members of the USC Biomedical Microsystems Laboratory for their assistance.

References

[1] A. B. Schwartz, X. T. Cui, D. J. Weber, and D. W. Moran, "Brain-controlled interfaces: movement restoration with neural prosthetics," *Neuron,* vol. 52, pp. 205-220, 2006.

[2] V. S. Polikov, P. A. Tresco, and W. M. Reichert, "Response of brain tissue to chronically implanted neural electrodes," *J Neurosci Meth,* vol. 148, pp. 1-18, 2005.

[3] W. He, G. C. McConnell, and R. V. Bellamkonda, "Nanoscale laminin coating modulates cortical scarring response around implanted silicon microelectrode arrays," *J Neural Eng,* vol. 3, p. 316, 2006.

[4] M. R. Abidian and D. C. Martin, "Multifunctional nanobiomaterials for neural interfaces," *Adv Funct Mater,* vol. 19, pp. 573-585, 2009.

[5] N. Han, S. S. Rao, J. Johnson, K. S. Parikh, P. A. Bradley, J. J. Lannutti, and J. O. Winter, "Hydrogel–electrospun fiber mat composite coatings for neural prostheses," *Front Neuroeng,* vol. 4, 2011.

[6] P. R. Kennedy, "The cone electrode: a long-term electrode that records from neurites grown onto its recording surface," *J Neurosci Meth,* vol. 29, pp. 181-193, 1989.

[7] J. S. Brumberg, P. R. Kennedy, and F. H. Guenther, "Artificial speech synthesizer control by brain-computer interface," in *Interspeech*, 2009, pp. 636-639.

[8] P. R. Kennedy and R. A. E. Bakay, "Activity of single action potentials in monkey motor cortex during long-term task learning," *Brain Res,* vol. 760, pp. 251-254, 1997.

[9] C. S. Hughes, L. M. Postovit, and G. A. Lajoie, "Matrigel: a complex protein mixture required for optimal growth of cell culture," *Proteomics,* vol. 10, pp. 1886-1890, 2010.

[10] K. Jin, X. O. Mao, L. Xie, V. Galvan, B. Lai, Y. Wang, O. Gorostiza, X. Wang, and D. A. Greenberg, "Transplantation of human neural precursor cells in Matrigel scaffolding improves outcome from focal cerebral ischemia after delayed postischemic treatment in rats," *Cerebr Blood F Met,* vol. 30, pp. 534-544, 2009.

[11] A. Passaniti, R. Taylor, R. Pili, Y. Guo, P. Long, J. Haney, R. Pauly, D. Grant, and G. Martin, "A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor," *Lab Invest,* vol. 67, p. 519, 1992.

[12] J. T. W. Kuo, B. J. Kim, S. A. Hara, C. D. Lee, C. A. Gutierrez, T. Q. Hoang, and E. Meng, "Novel flexible Parylene neural probe with 3D sheath structure for enhancing tissue integration," *Lab Chip,* 2013.

[13] L. Spataro, J. Dilgen, S. Retterer, A. Spence, M. Isaacson, J. Turner, and W. Shain, "Dexamethasone treatment reduces astroglia responses to inserted neuroprosthetic devices in rat neocortex," *Exp Neurol,* vol. 194, pp. 289-300, 2005.

[14] H. K. Kleinman and G. R. Martin, "Matrigel: Basement membrane matrix with biological activity," *Semin Cancer Biol,* vol. 15, pp. 378-386, 2005.

[15] R. B. Friedrich, A. Ravanello, L. C. Cichota, C. M. B. Rolim, and R. C. R. Beck, "Validation of a simple and rapid UV spectrophotometric method for dexamethasone assay in tablets," *Quim. Nova,* vol. 32, pp. 1052-1054, 2009.

[16] D. Szarowski, M. Andersen, S. Retterer, A. Spence, M. Isaacson, H. Craighead, J. Turner, and W. Shain, "Brain responses to micro-machined silicon devices," *Brain res,* vol. 983, pp. 23-35, 2003.

[17] C. S. Reinhard, M. L. Radomsky, W. M. Saltzman, J. Hilton, and H. Brem, "Polymeric controlled release of dexamethasone in normal rat brain," *J Control Release,* vol. 16, pp. 331-339, 1991.

[18] M. Uemura, M. M. Refaat, M. Shinoyama, H. Hayashi, N. Hashimoto, and J. Takahashi, "Matrigel supports survival and neuronal differentiation of grafted embryonic stem cell-derived neural precursor cells," *J Neurosci Res,* vol. 88, pp. 542-551, 2010.